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Novel Immunotherapeutic Approaches in Targeting Dendritic Cells with Virus Vectors

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Abstract: Viruses have evolved efficient strategies to overcome cellular membranes and transfer nucleic acid into a host cell. This property is being exploited in gene therapy which has the goal of delivering therapeutic genes into a patient tissue in order to achieve a clinically relevant effect. An interesting target for virus-mediated gene transfer is the immune system. In fact, the first human gene therapy trial performed involved the implantation of autologous bone marrow cells transduced *ex vivo* with gamma retrovirus vectors expressing adenosine deaminase in a patient with severe combined immunodeficiency. More recently, targeting transgene expression to dendritic cells (DCs) has become a promising strategy for directing the immune system towards immunity or tolerance. DC targeting has been achieved on a transcriptional level by using DC-specific promoters or by retargeting the tropism of the virus vectors. For example, we and others have developed strategies that support antigen-specific immune tolerance by transducing hematopoietic stem cells with lentivirus- or gamma retrovirus-vectors that transcriptionally target antigen expression to DCs. This review discusses the state of the art of vector-targeting to DCs in preclinical as well as clinical trials. [*Discovery Medicine* 20(109):145-151, September 2015]

Introduction

The immune system is constantly occupied with fighting and ultimately defeating pathogens, eliminating tumors, and re-establishing homeostasis. To avoid autoimmune disease, immune suppression mechanisms have to be in place to prevent the immune system from overreacting. In this context, dendritic cells play an important role in capturing and presenting antigens to T cells to induce immunity on one hand, and immune tolerance on the other hand.

The DCs are the most efficient professional antigen-presenting cells (APC) due to many features that contribute to regulating the immune system including (i) high levels of MHC class I and class II molecules that are constitutively synthesized; (ii) expression of accessory molecules responsible for T cell adhesion (ICAM-1) and co-stimulation (CD40, CD80 and CD86) (Scheeren *et al*., 1991; Larsen *et al*., 1992; Young *et al*., 1992); (iii) production of immunostimulatory cytokines that are up-regulated after recognition of pathogen-associated molecule patterns (PAMPs) or through endogenous signals released by dying cells; (iv) capability of migrating from inflamed tissue to lymph nodes due to down regulation of chemokine receptors such as CCR6 and CCR5 which are responsible for peripheral tissue localization and recruitment to inflamed sites, respectively (Luster, 2002), and up-regulation of CCR7 (Randolph *et al*., 2005) or CCR2 (CCR7-independent pathway) (Nakano *et al*., 2009) in monocyte-derived DCs and CCR8 in skin-derived DCs that allows their entry into lymph nodes (Randolph *et al*., 2005). (v) In addition, DCs are the most efficient APCs in cross-presenting exogenous antigens via MHC I to CD8+ T cells (den Haan *et al*., 2000; Heath *et al*., 2004; Joffre *et al*., 2012). This process favours cytotoxic T lymphocyte (CTL) activation against viral infections and tumor cells.

Indeed, for decades DCs have been considered highly effective tools for inducing robust and specific therapeutic immune modulation. A well-established approach of loading autologous DCs *in vitro* with tumor-specific peptides before re-injecting them into recipient organisms generated promising results both in pre-clinical trials in mice (Badovinac *et al*., 2005; Hira *et al*., 2015) as well as in human phase I/II trials with

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proven safety (Nestle *et al*., 1998; Dillman *et al*., 2012; Wang *et al*., 2015). The in vitro transduction of autologous DCs with viral vectors to "load" DCs with antigen has also resulted in efficient induction of antigen-specific cellular and humoral immune responses (Brossart *et al*., 1997; Song *et al*., 1997). Despite the promising results obtained from *ex vivo* manipulation of DCs, isolation and expansion of these cells is time consuming, laborious and the overall costs are high. Moreover, therapeutic effects of antigen-pulsed DCs would be restricted to patient's HLA and would depend on tumor availability (Jenne *et al*., 2001). Therefore, other approaches such as *in vivo* DC targeting or manipulation of DC-progenitors would be highly desirable. In this review we discuss promising strategies for targeting DCs with viral vectors in order to modulate the immune system.

Targeting DCs with Viral Vectors to Induce Immunity

The approach of using viral vectors to deliver genetic material into a cell and regulate the immune system has made significant progress in the last years. One of the advantages of using viral vectors for genetically modifying DCs is the concomitant induction of DC maturation (Schumacher *et al*., 2004). Human DCs transduced with recombinant adenovirus vectors for example up-regulate CD83 and down-regulate CD14, characterizing a mature phenotype, and the cells also down-regulate IL-10 production (Schumacher *et al*., 2004). A detailed description of the molecules involved in DC activation is found elsewhere (Apostolopoulos *et al*., 2013). The biggest challenge faced by *in vivo* transduction of DCs with viral vectors is targeting specific cells to assure safety and efficacy of immunization and reducing adverse effects in the surrounding cells. To achieve specific cell targeting, natural virus surface molecules (membrane glycoproteins, capsid proteins) are manipulated to create, enhance or eliminate the affinity to a specific cellular receptor. Cell surface receptors for targeting antigens to DCs include the C-type lectin receptors (DC-SIGN, DEC-205, dectins, mannose receptors), Toll-like receptors (TLRs) and other pattern recognition receptors (Palucka and Banchereau, 2012; Apostolopoulos *et al*., 2013).

The most important viruses that have been used as vectors in gene therapy protocols include adenovirus, adeno-associated virus, herpesvirus, poxvirus, and retrovirus (both standard gamma retroviruses and lentiviruses). These viruses transduce different cell types but can also be pseudotyped to redirect virus tropism. Here we discuss the state of the art of vector-targeting to DCs in preclinical as well as in clinical trials.

Induction of Immune Responses with Adenovirus Vector-modified DCs

Adenoviruses are non-enveloped, double-stranded DNA viruses with a transgene capacity of 7-35 kb. The virus genome is easily manipulated, and vector production yields high titers. Adenovirus vectors have been used in a wide range of clinical trials for inducing immune responses, including cancer vaccination approaches. The immunosuppressive tumor microenvironment results in poor activation of the immune system due to increased numbers of regulatory T cells and myeloid-derived suppressor cells, high levels of immunosuppressive cytokines, and induction of tumor antigen specific CD4+ and CD8+ T cell tolerance (Rabinovich *et al*., 2007; Shiao *et al*., 2011). Targeting tumor antigens to DCs may enhance tumor-specific effector T cell immune responses. However, the generation of a tumor-specific immune response has not always reflected in clinical response and improvement of clinical effects remains a challenge (Butterfield *et al*., 2008; Chia *et al*., 2012).

In a pilot phase I/II therapeutic vaccination strategy, patients with chronic hepatitis C virus infection received two doses of autologous monocyte derived DCs transduced with adenovirus vector (Zabaleta *et al*., 2015). The recombinant adenovirus vector encoded the hepatitis C virus NS3 protein and a chimeric molecule consisting of the coxsakie and adenovirus receptor (CAR) fused to the ectodomain of CD40L (CFh40L), enhancing DC transduction and maturation. Vaccination showed no side effects but the immune responses generated against NS3 protein were poor and not sufficient to control hepatitis C virus infection. T cell exhaustion has already been described in hepatitis C virus infected patients (Sumida *et al*., 2013) and may be an explanation for the lack of NS3-specific T cell priming. Future trials should also consider the impact of the adapter CFh40L that appears to increase IL-10-production by DCs. IL-10 may be involved in down regulating hepatitis C virus-directed cellular immune response (Zabaleta *et al*., 2015).

The low magnitude of antigen specific immunity induced by recombinant adenovirus vector transduced DCs highlights the importance of employing molecules that boost immune responses. A successful approach to enhance antigen delivery to DCs consists of linking antigen to monoclonal antibodies that specifically bind to DC receptors involved in antigen uptake. Indeed, in animal models (Idoyaga *et al*., 2011; Flacher *et al*., 2012) and *ex vivo* human cells (Dakappagari *et al*., 2006) or tissue (Stoitzner *et al*., 2014) it has been well established that targeting DC receptors such as

DEC205, Dectin-1, Langerin and other DC-specific molecules increases antigen uptake and presentation. Mice immunized with a replication-defective adenovirus vector encoding ovalbumin (OVA) fused to a DEC205-specific antibody resulted in enhanced CD8+ T cell proliferation and IgG production (Tenbusch *et al*., 2013). High doses of adenovirus vectors expressing OVA-DEC205 induced also CD4+ T cell responses that were not observed after vaccination with the adenovirus vectors encoding only OVA.

Induction of Immune Responses with Gamma Retrovirus Vectors

Retroviruses are enveloped, single-stranded RNA viruses that are able to integrate into the host cell genome as a provirus. With a transgene capacity of 7.5kb, retroviruses are among the viral vectors most frequently used in clinical trials, gamma retrovirus vectors in particular and more recently also lentivirus vectors. Studies that applied gamma retrovirus vectors in animal models and in human patients have shown promising results in ameliorating several genetic diseases, including X-linked severe combined immunodeficiency disorder (X-SCID) (Hacein-Bey-Abina *et al*., 2010), b-thalassemia (Negre *et al*., 2011), Wiskott-Aldrich syndrome (Aiuti *et al*., 2013) and metachromatic leukodystrophy (Biffi *et al*., 2013). Moreover, gamma retrovirus vector transduced hematopoietic stem cells (HSCs) have been shown to induce immune tolerance in animal models of autoimmune disorders (Xu *et al*., 2006; Chan *et al*., 2008; Eixarch *et al*., 2009). However, the limitation of using retroviruses in gene therapy is the risk of insertional mutagenesis. In fact, random genomic integration was demonstrated in a clinical trial for X-linked severe combined immunodeficiency disorder (X-SCID) in which 5 out of 20 patients developed leukaemia because of activation of the oncogene LMO2 (Howe *et al*., 2008; Hacein-Bey-Abina *et al*., 2010). It is still not known if the mutation occurred due to viral sequences or due to the transgene itself. In X-SCID patients the g-chain of the IL-2 receptor (IL2RG) is mutated. The retrovirus vectors were used to restore the expression of the IL2RG. According to Woods *et al*. (2006), the proliferative disorder of the T cells was caused rather by the IL2RG gene itself than by genomic insertion of the vector DNA. This conclusion was based on mouse experiments which showed that X-SCID mice developed T cell lymphomas after receiving HSCs transduced with lentivirus vectors carrying the murine IL2RG transgene, while control mice reconstituted with HSCs transduced with a control lentivirus vector did not (Woods *et al*., 2006)[176].

In contrast to gamma retroviruses, lentiviruses integrate preferentially in transcriptionally activate loci, thereby reducing the risk of proto-oncogene activation (Schröder *et al*., 2002). In addition, several modifications to the vectors have improved their safety profile. For example, self-inactivating (SIN) lentivirus vectors were constructed by deleting the promoter and enhancer elements from the U3 region of the viral genome, generating a replication-incompetent virus (Miyoshi *et al*., 1998). The deletion of the viral promoter allows the insertion of cell type-specific promoters that support transgene expression in specific cells and are therefore less likely to be silenced, thus improving transduction efficiency and safety of vectors. Moreover, as opposed to gamma retroviruses, lentiviruses can efficiently transduce not only dividing but also non-dividing cells including HSCs, which constitutes a major advantage particularly also for manipulating the immune system, as discussed in the following section.

Induction of Immune Responses with Lentivirus Vector-modified DCs

Because of the aforementioned properties, lentivirus vectors have been favoured over gamma retrovirus vectors in recent years. The sindbis virus envelope protein has been used for lentivirus vector pseudotyping to target DCs. Sindbis virus envelope glycoproteins recognize and bind preferentially to the DC-SIGN (CD209) surface molecule that is expressed mainly by immature and mature DC subpopulations (Geijtenbeek *et al*., 2000). However, sindbis virus envelope glycoprotein E2 can also bind to heparan sulphate (HS) which is present on the surface of many different cells. Yang *et al*. (2008) showed that more specific DC targeting can be achieved by deleting the HS binding site of E2. After subcutaneous injection in mice, pseudotyped lentivirus vectors efficiently transduced DCs, supported the migration of activated DCs to regional lymph nodes, and induced antigen-specific cellular immune response (Yang *et al*., 2008). Another approach to retarget lentivirus vectors to APCs consists of fusing single-chain antibodies that bind to MHC-II molecules with envelope proteins of the vector particles. Such vectors can efficiently activate CD8+ T cells in mice (Gennari *et al*., 2009).

In a cancer vaccination approach in mice, subcutaneous injection of lentivirus vectors expressing tumor-associated antigens or, as control, ovalbumin resulted in the efficient transduction of DCs *in vivo*. Moreover, transduced DCs were found in the draining lymph nodes and in the spleen, and induced antigen-specific CD8+ (Esslinger *et al*., 2003; Dullaers *et al*., 2006) and CD4+ T cell responses (Dullaers *et al*., 2006). Anti-tumor-specific immune responses also contributed to tumor

growth inhibition and prolonged survival of the animals after challenge (Dullaers *et al*., 2006).

Transgene expression levels should also be considered when choosing a strategy to target DCs. Although the dendritic cell-associated C-type lectin, the dectin-2 promoter, restricts transgene expression to Langerhans cells in the skin and CD11c+ populations in the lymph nodes and induces antigen-specific CD8+ and CD4+ T cell responses in mice, the low levels of transgene expression can compromise therapeutic effects (Lopes *et al*., 2008).

A promising approach is to genetically modify and reprogram DC precursors by lentivirus vectors. In that context, murine HSCs have been transduced in vitro with a tricistronic lentivirus vector that co-expresses granulocyte macrophage colony stimulating factor (GM-CSF), interleukin 4 (IL-4) and the herpes simplex virus-thymidine kinase (HSV-TK) gene (Pincha *et al*., 2011; 2012). The co-expression of GM-CSF and IL-4 by vector-transduced HSCs was used to drive in vitro cell-differentiation into self-differentiated myeloid-derived antigen-presenting-cells reactive against tumors (SmartDCs) while the suicide gene HSV-TK allowed *in vivo* depletion of transduced cells upon ganciclovir treatment. SmartDCs transduced with viral vectors that encoded melanoma related antigen induced therapeutic improvement in mice with reduced tumor growth and higher survival rate compared to the control group (Pincha *et al*., 2011).

Induction of Immune Tolerance by Virus Vector-modified DCs

DCs have not only the function to boost an immune response, but also to shut it down when over-reactive (Figure 1). Injection of immature blood monocyte precursor derived DCs pulsed in vitro with antigens into two healthy volunteers resulted in antigen specific cellular immune tolerance characterized by a decrease of IFNg-producing T cells and an increase of IL-10-producing T cells in a small randomized study (Dhodapkar *et al*., 2001). However, using virus vectors for targeting antigen presentation to DCs without inducing DC stimulation/maturation can be challenging. Conflicting results have indeed been published regarding induction of DC maturation by lentivirus vectors (Gruber *et al*., 2000; Breckpot *et al*., 2007; Toscano *et al*., 2010). In a recent study it was demonstrated that lentivirus vectors induced maturation of plasmacytoid DCs while the phenotype of myeloid DCs and in vitro differentiated monocyte-derived DCs was not altered (Rossetti *et al*., 2011). Using a lentivirus vector expressing vasoactive

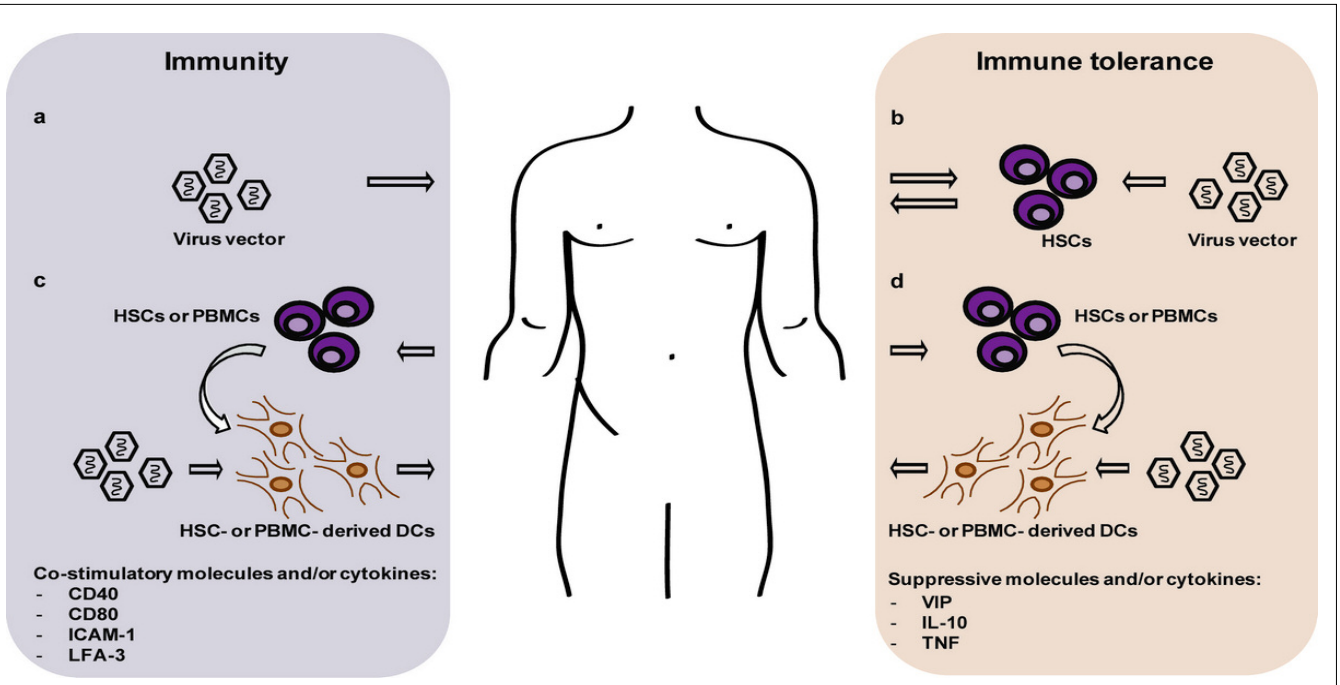

Figure 1. Current protocols for physically or transcriptionally targeting transgene expression to DCs. (a) In vivo DC targeting by direct injection of virus vectors to boost antigen-specific immune responses. (b) Injection of hematopoietic stem cells (HSCs) transduced in vitro with virus vectors to induce antigen-specific immune tolerance. (c and d) Injection of HSC-derived DCs or peripheral blood mononuclear cell (PBMC)-derived DCs transduced in vitro with viral vectors to induce (c) immunity or (d) tolerance, depending on co-expression of stimulatory or suppressive signals.

intestinal peptide (VIP) that has immune suppressive activity, Toscano *et al*. (2010) showed that mice injected with myelin protein loaded lentivirus vector-transduced BM-derived DCs were protected from experimental autoimmune encephalomyelitis (EAE). Furthermore, in an asthma model in mice, bone marrow (BM)-derived DCs transduced with recombinant lentivirus vectors encoding OVA antigen and IL-10 were able to induce OVA-specific immune tolerance (Henry *et al*., 2008).

Considering that the therapeutic effect of DCs transduced *ex vivo* with lentivirus vectors will be restricted by the life span of those cells, a feasible approach to induce long-term expression of specific antigens by steady state DCs is the manipulation of DC progenitors, the HSCs.

In this context, our group and others demonstrated that the transduction of HSCs with SIN-lentivirus vectors (De Andrade Pereira *et al*., 2013; 2015) or SIN-gamma retrovirus vectors (Ko *et al*., 2011) resulted in efficient antigen-specific immune tolerance in mice.

Although, lentivirus vectors are more efficient in transducing quiescent cells (Naldini *et al*., 1996) such as HSCs, targeting myelin oligodendrocyte glycoprotein (MOG) expression to dendritic cells with a gamma retrovirus vector significantly delayed the onset of EAE in mice compared to controls (Ko *et al*., 2011). In this study MOG was under transcriptional control of a 960bp fragment of the CD11c promoter (-960 to +51 bp) and the transgene was expressed indeed predominantly in CD11c+ MHC-II+ DCs. Absence of protection from EAE in that study could be related to low level of antigen expression due to the promoter or inefficient transduction of HSCs or both.

In order to restrict transgene expression to DCs, our group transduced BM-derived HSC with a SIN-lentivirus vector that expresses MOG under transcriptional control of the DC-specific transmembrane protein (DC-STAMP) promoter (Figure 2). The DC-STAMP promoter resulted in transcriptional targeting of transgene expression mainly to DCs (De Andrade Pereira *et al*., 2013). All mice, which received HSCs transduced with this vector were fully protected from EAE induced either by immunization with myelin antigen (De Andrade Pereira *et al*., 2013) or by transfer of myelin antigen-reactive T cells (De Andrade Pereira *et al*., 2015). Protection was concomitant with the generation of MOG-specific regulatory CD4+ T cells and depletion of MOG-specific CD4+ T cells (De Andrade Pereira *et al*., 2013). Importantly, tolerance was induced towards the specific self-antigen MOG while T cell reactivity to unrelated foreign antigens was fully preserved.

The adoptive transfer of myelin antigen-reactive T cells resulted also in the complete protection of tolerized animals from EAE, while non-tolerized mice rapidly developed disease symptoms (De Andrade Pereira *et al*., 2015). Tolerance induction correlated with the up-regulation of molecules associated with T cell anergy and regulatory T cell phenotype. The ability to revert the pathogenic phenotype of MOG-specific CD4+ T cells highlights the potential of this strategy as a therapeutic approach in an established autoimmune disease.

In our study, animals that received lentivirus vector transduced HSCs continued to produce MOG-expressing, tolerizing DCs for up to six months after transplantation (De Andrade Pereira *et al*., 2013). However, long-term transgene expression in human DCs remains to be assessed. In case of transgene silencing, modifications to the vector, such as the incorporation of scaf-

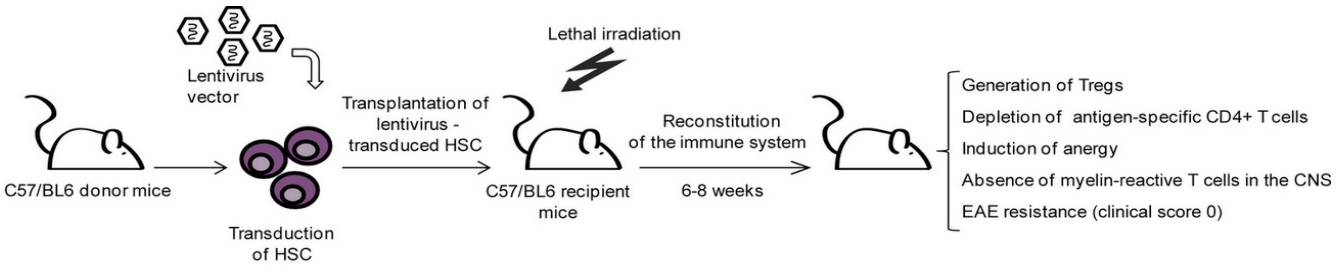

Figure 2. Induction of antigen-specific immune tolerance by lentivirus vector-transduced BM-HSC in an experimental autoimmune encephalomyelitis (EAE) model. HSCs are collected from 5-fluorouracil (5-FU) treated C57BL/6 donor mice and cultured in presence of cytokines. After three days HSCs are transduced with lentivirus vector and transplanted into lethally irradiated C57BL/6 recipient mice. Six to eight weeks after transplantation chimeras are challenged by immunization with myelin antigen or injection of activated myelin-reactive CD4+ T cells and between 7 and 14 days later analyzed for tolerance induction.

fold/matrix attachment regions or insulators to diminish the suppressive effect of nearby heterochromatin may be considered (Ramezani and Hawley, 2010).

Ongoing Clinical Trials Using Autologous DCs for Inducing Immune Responses

In most of the ongoing clinical trials DCs are modified *ex vivo* with non-viral methods, including peptide loading and mRNA transfection (Table 1). In fact, to this date, Provenge (Sipuleucel-T), a treatment for metastatic prostate cancer, is the only product based on autologous DCs that received marketing authorisation by the Food and Drug Administration (FDA). The treatment consists of culturing and activating peripheral-blood mononuclear (PBMC) APCs from patients with recombinant prostate protein (prostatic acid phosphatase) fused to GM-CSF (Kantoff *et al*., 2010a). In a double-blind, phase III trial, survival rate was prolonged in an average of 4.1-months in cancer patients treated with Sipuleucel-T as compared with the group that received placebo (Kantoff *et al*., 2010a).

Table 1. Overview of DC-based Clinical Trials.

Vehicle	Antigen	Disease	Phase	Status
mRNA-transfected autologous DCs	Autologous HIV mRNA transcripts	Chronic HIV infection	I/II	C
	mRNA encoding gp100 and tyrosinase	Uveal melanoma	I/II	R
Antigen loaded autologous DCs	Heat-inactivated autologous HIV	HIV infection[1]	II	C
	Autologous tumor antigens	Sarcoma	I/II	R
		Melanoma	III	R
		Metastatic colorectal neoplasm	II	C
		Brain tumor	II	C
		Advanced melanoma	I/II	C
	HIV-1 Lipopeptides; DC activation with LPS	HIV-1 infected patients	I	C
DEC-205/NY-ESO-1 fusion protein with or without rflt3 ligand	NY-ESO-1 & neoantigen-based melanoma[2]	Stage IIB-IV melanoma	II	R
DEC-205/NY-ESO-1 fusion protein and IDO1 inhibitor	DEC-205/NY-ESO-1 Fusion Protein CDX-1401	Cancer in remission[3]	I/II	R

Note: 1, very early stages of the disease; 2, neoantigen-based melanoma-poly-ICLC; 3, ovarian, fallopian tube or primary peritoneal cancer.
Abbreviations: C, completed; R, recruiting; DEC-205, endocytosis-mediating C-type lectin receptor; HIV, human immunodeficiency virus; gp, glycoprotein.

Table 2. Overview of DC-based Clinical Trials Using Virus Vectors.

Vehicle	Antigen	Disease	Phase	Status
Direct injection of adenovirus vector	GM-CSF	Non muscle invasive bladder cancer	II/III	O, NR
Direct injection of hybrid lentivirus vector (sindbis virus envelope)	NY-ESO-1	Advanced or metastatic cancer[1]	I	O, R
Autologous DCs transduced with adeno-associated virus vector	CEA	IV Gastric cancer	I	O, NR
Autologous DCs transduced with adenovirus vector	CAIX fused to GM-CSF	Metastatic kidney cancer	I	O, R
	NS3	HCV patients	I/II	C
	Her-2	Metastatic breast cancer	I	C
	p53	Extensive-stage small cell lung cancer	I/II	C
Autologous DCs transduced with vaccinia- or fowlpox virus vector	MUC-1	Liver/lung metastases from colorectal cancer	II	C

Note: 1, melanoma, sarcoma, ovarian, breast, bladder or non-small cell lung cancer.
Abbreviations: O, ongoing; C, completed; R, recruiting; CAIX, Carbonic anhydrase IX; GM-CSF, Granulocyte-macrophage colony-stimulating factor; CEA, Carcinoembryonic antigen; CSF, cerebrospinal fluid; NS3, Nonstructural protein 3; HCV, Chronic hepatitis C virus; Her-2, human epidermal growth factor receptor 2; MUC-1, mucin 1.

However, although not yet FDA approved therapies, there are several ongoing clinical trials that use virus vector transduced DCs for inducing immune responses (Table 2 and https://clinicaltrials.gov). For example, in a phase II randomized trial metastatic prostate cancer patients received a single dose of vaccinia virus vector followed by six boosts of fowlpox virus vectors both expressing a prostate-specific antigen and co-stimulatory molecules (CD80, ICAM-1 and LFA-3) (Kantoff *et al*., 2010b). All injections were accompanied by GM-CSF as an adjuvant. Control groups received empty viral particles. Survival improvement was observed in treated patients in a median rate of 8.5 months pushing the study forward to a phase III ongoing trial (clinical trial identifier number NCT01322490).

Another study in metastatic kidney cancer patients currently under phase I, involves autologous DCs transduced with a recombinant adenovirus vector encoding GM-CSF fused with a kidney cancer-associated antigen (AdGMCAIX; clinical trial identifier number NCT01826877). The efficiency of this approach was previously demonstrated in animals, in which mice transplanted with vector transduced DCs generated antigen-specific T cell immune responses and showed tumor growth inhibition (Birkhäuser *et al*., 2013).

An ongoing phase I trial in advanced and metastatic cancer patients uses sequential and direct injection of an integration-deficient hybrid lentivirus vector targeting the DC-SIGN receptor through sindbis virus envelope glycoprotein pseudotyping and expressing a highly immunogenic tumor antigen NY-ESO-1 and a second injection of full length NY-ESO-1 protein together with a TLR4 agonist (clinical trial identifier number NCT02387125). This approach has the advantage that it avoids *ex vivo* manipulation of patient cells.

Conclusion

Many studies in animal models demonstrated the potential of DCs for either enhancing antigen specific cellular immune responses or immune tolerance. Clinical trials in human patients with antigen-pulsed-, mRNA transfected- or virus vector modified DCs proved safe and were associated with only mild side effects. Recent studies showed that clinical improvement may be achieved by combining DC transduction with immunomodulation. For cancer treatment for example, antigen expression/presentation by DCs could be combined with blocking of negative regulatory molecules such as PD1/PDL1 and CTLA-4 in order to circumvent T cell exhaustion and induce an efficient tumor specific immune response (Metcalfe *et al*., 2015). When the aim is to induce a broad immune response against several antigens in an HLA-independent manner, virus vectors would be the prime choice. DCs modified by viral vectors expressing full-length proteins activate not only CD4+ T cells but induce also antigen cross-presentation to CD8+ T cells. In an autoimmune context, the strategy of expressing full-length protein as antigen may allow to induce immune tolerance against multiple epitopes and avoid epitope spreading. A gene therapy approach that combines the genetic modification of DCs with virus vectors and the manipulation of HSCs may efficiently induce self-protein tolerance in absence of generalized immunosuppression. Future strategies for livelong interventions in the immune system in genetic and autoimmune diseases should consider targeting virus vectors physically to HSCs but transcriptionally to DCs. However, the long-term effects of targeting DCs with virus vectors are unknown and should be considered with caution.

Recent pre-clinical and clinical trials using virus vector-modified DCs for regulating the immune system have yielded promising results. Nevertheless, there is still room for optimizations concerning DC targeting as well as the induction of potent antigen-specific immune responses while avoiding vector specific host responses. Moreover, vector safety may be improved by incorporating suicide genes that allow the elimination of vector transduced cells in case of adverse effects.

Disclosure

The authors state no conflicts of interest.

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